

The Chromosome Complex of *Culex pipiens*.

Part II.—Fertilisation.

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With Plate 20 and 1 Text-figure.

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INTRODUCTION.

IN the summary of a paper, entitled "*The Chromosome Complex of *Culex pipiens**" (4), it was stated that:

(1) The somatic number of chromosomes is three, both in the male and female.

(2) The number of chromosomes in the spermatogonia, as well as in the primary and secondary spermatocytes and spermatids, is three.

Two alternative suggestions as to the cause of this apparent anomaly were offered:

(1) The non-participation of one of the gametic nuclei in the formation of the "zygote" in "fertilisation."

(2) The fusion of three pairs of homologous chromosomes at some early stage in the life-history, this fusion remaining permanent throughout later divisions. In the discussion preceding the summary it was stated that the question could not be settled until the fertilisation process had been examined.

This work on the cytology of *Culex*, as stated in the introduction of the above paper, had been undertaken because of the importance of the conclusions given by Miss Stevens in her paper, entitled "The Chromosomes in the Germ-cells of *Culex*" (3).

The second of the alternative suggestions offered above is a modification to meet the particular needs of the case of *Culex pipiens* of Miss Stevens' statement that: "Parasynapsis (parasynopsis) occurs in *Culex* in each cell generation of the germ-cells, the homologous maternal and paternal chromosomes being paired in telophase, and remaining so until the metaphase of the next mitosis."

Difficulties in obtaining the necessary material, and in the technique connected with the food supply of the imagines, delayed, for the time being, the examination of the fertilisation processes, and prevented the demonstration of the whole history of oogenesis.

Dr. Woodcock's paper "On 'Crithidia' fasciculata in hibernating mosquitoes (*Culex pipiens*) and on the question of the connection of this parasite with a Trypanosome" (5) has, however, incidentally filled up some of the gaps left in the history of oogenesis, and, in this paper, the technique of artificial rearing of *Culex pipiens* in all stages of its life-history has been described.

I should like to take this opportunity of thanking Dr. Woodcock not only for sending me a Reprint of this paper, but also for giving me full details in writing of his experiments, by which I have been enabled to repeat his work with similar success, and also, as will be shown later, to confirm his statement that "*Culex pipiens* is essentially the British mosquito which likes Avian blood."

In the 'Proceedings of the Royal Society,' 1915, a full description of the chromosome cycle of Coccidia and Gregarines appeared (Dobell and Jameson) (1), a work which elucidates a cytological condition of affairs superficially somewhat similar to that which obtains in *Culex pipiens*. The authors of that paper demonstrate the presence of the haploid number of Chromosomes at every nuclear division in the life-history of *Aggregata* and *Diplocystis* except in the zygote.

In 'Chromosome Studies of Diptera' (2), July, 1914, Charles W. Metz calls attention to the neglect of Diptera by students of cytology—a neglect all the more pronounced when contrasted with the great amount of energy expended upon other insect-groups—notably Hemiptera, Orthoptera, Coleoptera. He offers, as a probable explanation of this neglect, the unsuitability of Dipterous material for cytological study and the great difficulties connected with such study. The results embodied in Metz's paper, as will be shown later, have been helpful in interpreting the phenomena observed in *Culex* and in showing that there is no essential difference between it and other Diptera.

Dr. Woodcock discovered that, after the summer female of *Culex pipiens* has fed once on the blood of a living bird, the eggs attain their normal size and are ready for fertilisation. The raft is laid almost immediately after the second feed—fertilisation taking place in the interval between the two meals. This probably accounts for the fact that females reared in captivity and fed on a fruit diet appear never to be fertilised.

MATERIAL AND METHODS.

In the summer of 1915 cages similar to those described by Dr. Woodcock were set up in the College Laboratory of Notre Dame, Glasgow, young pigeons being employed as food. A pigeon was also caged in the near neighbourhood of a wooden tub stocked with larvæ, and placed in a small garden at Notre Dame. Control experiments, which will be described

later, were also started at Ladywood, Milngavie, the source of the material for all these experiments being mainly a rectangular iron trough near the farm-yard of Garscadden Mains, Bearsden. Two rafts were found during the course of the summer 1915 in the wooden tub, although all attempts in previous years to induce the imagines to lay there, or to lay in any of the aquaria on the premises, had failed. This experiment would seem to show that wild birds are not so easily bitten by the gnats as domestic birds, since many sparrows, thrushes, and blackbirds visit the small garden in which the tub is situated.

The season's experience of artificial rearing showed that the number of egg-rafts produced under artificial conditions was not likely to be sufficient for a thorough investigation of the fertilisation processes. Moreover, unfertile egg-rafts were frequently obtained by Dr. Woodcock from the artificially confined imagines, and I too obtained rafts which produced no larvæ; while, on the other hand, egg-rafts laid in the open invariably produced larvæ. For these reasons the confinement of the imagines in netted chambers was abandoned, and the stocking of a pond at a convenient place in the country was resolved upon.

In view of the above experiments, and of the fact that all the sources of material already used had been situated in the vicinity of farm-yards, it was decided to establish such a pond at Ladywood, near a stock of poultry—these latter birds being evidently easier of access than wild birds. Earlier in the summer of 1915 several small troughs, 12 in. in diameter, and about 6 in. in depth, had been stocked with larvæ to serve as control experiments to those being carried on at Notre Dame. The results were disappointing. No egg-rafts appeared on these small ponds (during the summer of 1915). The number of female gnats seeking for hibernating quarters in the autumn of that year around Ladywood showed that the imagines, developed from the contents of the small troughs mentioned above, must have found a pond more suitable to the needs of their offspring than those pre-

pared for them in the garden of Ladywood. Mr. MacDougall's permission having been obtained, a hunt over his nursery gardens revealed the secret of the non-appearance of rafts on the Ladywood ponds. A large disused iron bath, elliptical in form (36 in. $\times$ 26 in. $\times$ by 11½ in.), and containing stagnant water, was swarming with larvæ and pupæ of every age which were evidently supplying hibernating imagines. This discovery was made too late in the year to be utilised for the further production of rafts—but it showed that the situation was a good one—the former experiments having possibly failed because of the smallness of the troughs, and because they were more or less concealed by the surrounding grass.

Early in 1916, thanks to the kindness of Mr. MacDougall, four ponds, A, B, C, and D respectively, were prepared: One (A), a large circular iron trough, diameter 30 in., greatest depth 24 in.; another (B), a wooden tub, 25 in. in diameter, depth 16 in.; the third (C) was the bath already selected by the gnats in 1915; the fourth (D), a rectangular porcelain sink, 20 in. $\times$ 14 in. $\times$ 10 in.

The first two, A and B, were situated side by side, being protected on the north by shrubs, and on the east by a wall. The iron trough (A) was exposed to all the sunshine of morning, afternoon, and evening; the wooden one (B), being nearer the wall, was more shaded. Both, however, were quite unhidden by vegetation. The tinned iron bath (C) was surrounded on three sides by glass-houses, and exposed on the southern side; the fourth (D) was placed in the uncult grass of an open space.

On May 21st, 1916, fifteen egg-rafts were discovered in A—there was thus no necessity to stock the ponds. No rafts appeared on the water in the wooden pond until June 15th. The preference shown by the gnats for the iron trough, and for the tin bath, may be due to the higher temperature of the water of these, or to the fact that they were in a more exposed situation, and consequently more easily found. The porcelain trough has never been popular, comparatively few rafts being forthcoming.

The rafts found in May and June were evidently those laid by the hibernating imagines seen in the previous autumn, since, after that date, eggs were not abundantly produced until the middle of July, when a spell of exceptionally warm, moist, weather conduced to an abnormally abundant supply. This supply continued to be good until the middle of August, when the spell of hot weather ceased. Odd rafts were occasionally found until the end of August.

In one of his letters Dr. Woodcock expressed his opinion that the rafts were deposited at about 5 o'clock in the morning, and text-books also state that the early hours of summer mornings are chosen by the gnats for the purpose of egg-laying. On July 12th, 1915, an egg-raft, cream-white in colour, was found at the Bearsden pond at 5 a.m. Portions of this raft were fixed at intervals of a quarter of an hour, and sections showed that it was quite young. After a careful study of ponds A, B, C, and D it became clear that, at Milngavie, most rafts are laid between the hours 9.30 p.m. and 12 p.m., very few between 12 p.m. and 4 a.m., and some between 4 a.m. and 6 a.m. As the season advances fewer are laid in the morning hours. Damp heat conduces greatly to the deposition of rafts, many more being forthcoming after a day of calm, moist, close weather. Numerous imagines were always hovering over the ponds in the evenings at a height of about six feet. These disappeared during the day time.

No difficulty in rearing the larval and pupal stages of the gnat occurred until the summer of 1915—the critical period in the life-history of the *Culex* being apparently the imago period. Even in exceptionally favourable circumstances the number of egg-rafts deposited at any one period bears a very small proportion to the number of imagines that escape from the pupal cases, and this has been very marked in a long study of the naturally occurring ponds. However, in the summer of 1916 *Daphnia* were introduced into the gnat cultures at Notre Dame, and since these, like the *Culex* larvæ, also flourish well on a diet of *Chlamydomonas*, the tub quickly became swarming with *Daphnia*, while the gnat

larvæ disappeared almost entirely. Perhaps this accounts for the more frequent occurrence of *Culex* in the fairly clean water of rain-barrels and drinking-troughs, in spite of the fact that, as shown by long experience, the larvæ grow more quickly in a good culture of *Chlamydomonas*, and that, on this account, one would expect the gnats to prefer a more stagnant habitat.

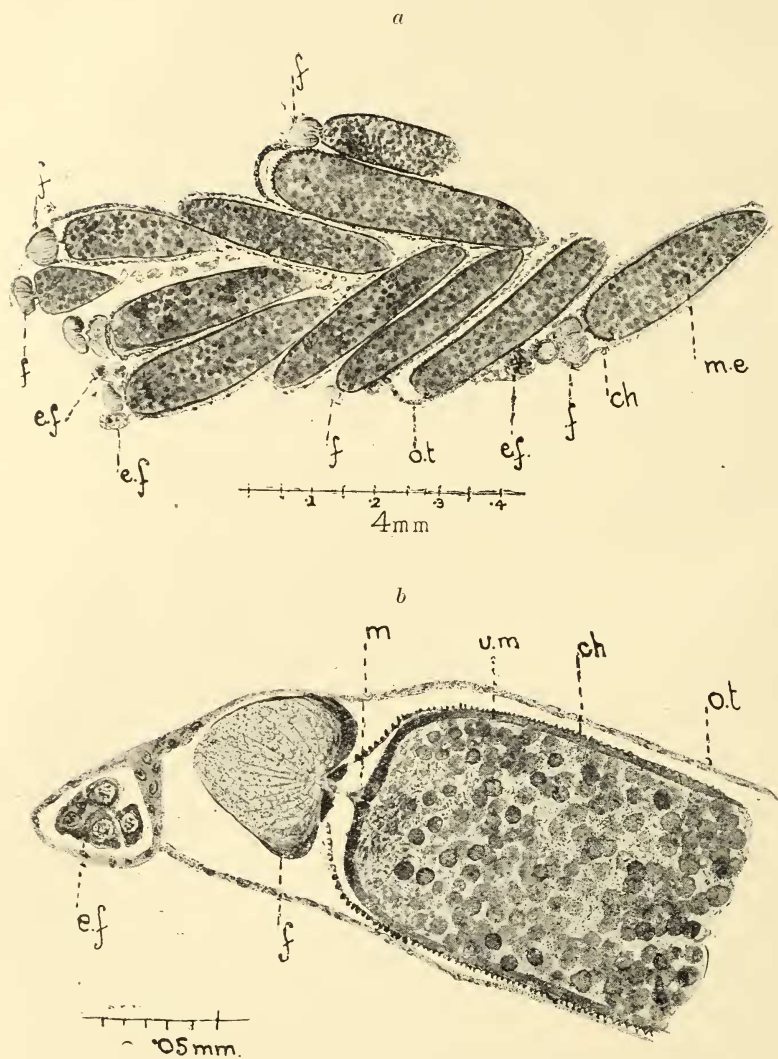
The egg-rafts, for the cytological studies in question, after being laid, which process takes about ten to fifteen minutes, were isolated, along with sufficient water from the pond, in Petri dishes, in which they underwent their subsequent development. Thus it was possible to determine the age of the eggs. Rafts just laid, and of ages ranging from half hour after the deposition of the last egg to two and three-quarter hours, were preserved. Imagines just before, during, and after oviposition were also fixed. Many rafts of unknown age were necessarily found during the season, their colour indicating to a certain extent their age.

My best thanks are offered to Sr. Carmela, S.N.D., who, during my absence in August, went on with the work of tending the ponds and fixing the rafts at timed intervals. She gives twenty minutes as the period required for the laying of one raft.

Carnoy proved an excellent fixative for both rafts and imagines. Bouin and Gilson-Petrunkewitsch were used as controls. The rafts did not sink in the former fixative, hence the results were not so good, but the Bouin material was more easily sectioned. Portions of whole rafts were embedded in paraffin, and sagittal sections of from 5μ to 8μ made of the whole mass. Thus many eggs could be examined at once. The funnel (see Text-fig. 1, *f*) was removed before embedding.

Mr. P. Jamieson's long experience in microtoming was placed entirely at my disposal in the matter of sectioning the proverbially difficult dipterous egg, and I should like to express my gratitude to him for this help, and also for actually cutting many of the sections used in this investigation.

TEXT-FIG. 1.



Sections through mature female gnat parallel to the sagittal plane (*b* under higher magnification). *ch.* Chorion. *e.f.* Young egg follicle. *f.* Funnel. *m.* Micropyle. *m.e.* Mature egg. *o.t.* Ovarian tube. *v.m.* Vitelline membrane.

The Reproductive Organs in the younger stages have already been described. In the mature ovary (Text-fig. 1) the egg is elongated, and is provided with a chorion, very beautifully sculptured, and a second membrane—the vitelline membrane—both of which are perforated before fertilisation by the micropyle. Separating one egg from its neighbour of the same size, or from a young egg follicle, is a chitinous structure (see fig. 30, 'Natural History of Aquatic Insects,' Miall.), like a funnel in shape, which remains attached to the laid egg at its broad anterior end. This funnel is perforated above the micropyle, and apparently serves to guide the spermatozoa to the micropyle. A somewhat similar structure is shown in Sedgwick's 'Student's Text-book of Zoology,' vol. 3 (fig. 400), for the egg of *Drosophila cellaris*. The future head end of the embryo lies in the anterior position in the ovarian tube, as is usual in insects—the hind end of the egg being the first to emerge from the imago.

Dr. Woodcock (5) expressed his opinion that an imago was probably capable of depositing more than one raft in the season. Sections of gnats, which were fixed immediately after depositing their rafts, showed that the contents of the spermathecae were by no means exhausted. Moreover, the gonad showed some large eggs, as well as very many small egg follicles, which evidence seems to support his opinion. The gnats, flying away after laying eggs, were perfectly vigorous, and showed no tendency to die.

The three spermathecae communicate with the hind end of the common oviduct by three minute tubes, so that the spermatozoa make their way into the funnel as the egg passes out.

In view of the foregoing description and the fact that eggs laid in captivity often produce no larvæ, the first hypothesis as to the origin of the haploid number of chromosomes in the somatic tissues of *Culex* can be rejected, and this is justified by actual observation of the two pronuclei in the egg.

The Egg.—I have not been able to observe the formation

of any polar bodies. The egg nucleus lies in the middle of the egg, and is a typical resting nucleus. It is surrounded by the large yolk globules which constitute the greater bulk of the egg. The cytoplasm, which is sparse, is densely staining and provided with numerous deeply-stained particles, as was the case in the tissues of the larva and pupa, and there are large numbers of small bodies surrounding the yolk globules which resemble chromatin in their staining reactions. These chromatin-like bodies, "yolk nuclei", seem to be intimately concerned with the digestion of the globular yolk-masses, and with their conversion into protoplasm, and sometimes resemble very minute chromosomes (Pl. 20, fig. 1). When surrounded by the digestion products, which have resulted from their activity, they do not stain so clearly, and eventually they appear in the fully-formed protoplasm as the particles already described. This quality of the protoplasm greatly detracts from the beauty of the histology.

The sperm nucleus (male pro-nucleus) assumes the resting condition very quickly. In one egg of a raft, fixed a few minutes after deposition, the two pro-nuclei are already in close contact. In one egg of a raft half an hour old, the spermatozoan has reached the egg nucleus, but is still sperm-like. In one-hour rafts there are several segmentation nuclei. (It must be remembered that there is a difference in age between the first laid egg of the raft and the last laid egg, of from ten to twenty minutes, hence these times are only approximate.)

The segmentation nuclei, which lie in little islands of protoplasm (Pl. 20, fig. 2) pass into a decided resting-stage after dividing, and several blocks have to be sectioned to make sure of obtaining mitotic nuclei.

The prophases (Pl. 20, figs. 4-9) of the dividing segmentation nuclei of *Culex* show six chromosomes. In early prophase a tendency to emerge in pairs from the resting nucleus is evident (Pl. 20, fig. 9). These six chromosomes (Pl. 20, fig. 10) arrange themselves on the equator of a perfectly typical spindle, split longitudinally, and in anaphase

and telophase six thin chromosomes can be seen going to each daughter nucleus (Pl. 20, figs. 11-13). In contrast to former experience of larval, pupal, and imaginal material the dividing nucleus is most frequently found in the metaphase and anaphase stage in the segmenting egg of *Culex*. What takes place in late telophase and in the passage of the chromosomes into the resting nucleus reticulum is difficult to follow.

There is thus no difficulty in demonstrating six chromosomes in the segmenting nuclei of *Culex pipiens*—fertilisation and the early stages of development are perfectly typical. No reduction in the “Zygote,” as found by Dobell and Jameson in *Coccidia* and *Gregarines*, takes place. The second hypothesis set forth in the beginning, viz. “the fusion of three pairs of homologous chromosomes at some early stage in the life-history, this fusion remaining permanent throughout later divisions,” most probably explains the case. Early prophases show decided tendency of the chromosomes to come out of the reticulum in pairs, full prophases show six chromosomes, among which pairing can sometimes be detected (Pl. 20, figs. 4, 7, 8, 9). As has been shown by Stevens and Metz a side-to-side pairing of homologous chromosomes is a characteristic of Dipterous cytology. Metz (2) states that Miss Stevens records it in nine species of *Muscidæ*, and four species of Mosquitoes, and that he has verified it in five of these species of *Muscidæ*, and extended it to eight others, in addition to species of *Drosophila*. An increasingly closer proximity of these homologous chromosomes one to the other, would produce, eventually, an actual fusion of the maternal and paternal constituents, with the result, in the case of *Culex pipiens*, that in full prophase three, and not six, would be the apparent chromosome number. The three chromosomes that appear so persistently in the somatic tissue of larvæ, pupæ, and imagines of *Culex pipiens* are, therefore bivalent in character; they are really three groups of chromosomes, with two in each group. I have not been able to trace out more fully the development of this tendency

of homologous chromosomes to fuse into an apparently single chromosome. This would entail a study of rafts ranging from a quarter hour to three and a half to four days old. (The development of the larva in Scotland occupies from three and a half to four days.) Moreover, the egg capsule becomes so hard and brittle as it changes from cream white to dark brown, that a special technique would have to be devised for sectioning these eggs without destroying the nuclear detail. All that can be stated with certainty up to the present is, that the homologous chromosomes have not fused in segmenting nuclei—while this fused condition has become a characteristic of *Culex pipiens* in the early larval stage.

It would seem, then, that parasyn-desis has reached its limit in the somatic tissues of *Culex pipiens*, resulting in the actual fusion of homologous chromosomes, and that extreme parasyn-desis is responsible for the apparent anomaly described in the 'Chromosomes Complex of *Culex pipiens*', I.

GENERAL.

There is general agreement that we are justified in assuming (1) that hereditary qualities are represented by material substance, and (2) that this substance is either chromatin or is inextricably involved in chromatin. Granting these two assumptions, we seem logically bound, by the generally occurring accurate longitudinal splitting of the chromosomes in mitosis, to admit that the patches of material representing definite hereditary qualities are arranged in linear fashion along the course of the chromosome or thread of chromatin. But this involves necessarily a tendency of the hereditary substance representing one particular quality, or group of qualities, to segregate together. In other words, there must be, in the case of hereditary substance, an attraction of like for like. If this be so, there will be a tendency for chromosomes composed of corresponding patches of hereditary material arranged in like order, to come

together side by side with homologous poles together, the opposite of what happens in the case of magnetic attraction.

Such a hypothesis will account for the frequent occurrence of parasyndesis, and for the further accentuation of this into complete fusion as is supposed to take place in *Culex pipiens*.

SUMMARY.

(1) The egg-rafts of *Culex pipiens* are laid most copiously between hours 10.30 p.m. and 12 p.m. They are also laid between 4 a.m. and 6 a.m.

(2) Fertilisation in *Culex pipiens* is normal. Segmentation begins in less than an hour after the deposition of the last egg.

(3) The chromosome number in the segmenting nuclei is six.

(4) A tendency to parasyndesis is exhibited by the segmenting nuclei.

(5) Parasyndesis probably effects the condition of the chromosomes in the nuclei of larva, pupa, and imago, i.e. is responsible for the presence of the apparently "haploid" character of the nuclei in the somatic cells.

NOTE.—After the completion of the foregoing paper, Metz's "Chromosome Studies on the Diptera II. The Paired Association of Chromosomes in the Diptera, and its Significance", 'Journ. Exper. Zool.', xxi, came into my hands.

With regard to certain criticisms passed on my work, I should like to state :—

(1) The fixatives employed by me were precisely those employed by Metz (see p. 379, 'Quart. Journ. Micros. Sci.', vol. lx).

(2) Although I have not specified this, all the precautions recommended by Metz to secure good fixation and penetration were employed by me. I cannot, therefore, put my results down to bad fixation.

(3) I have failed absolutely in my former work on *Culex*, although I devoted much time and study to this point, to find any figures resembling Metz's figs. 166 and 167, or Stevens's figs. 8, 9, and 10. This failure may be attributed to differences in the material studied. It could not be due to bad fixation.

(4) Setting aside the Diptera, my figs. 68 and 69 (4), would be interpreted, by the vast majority of cytologists, as a split spireme, as chromosomes precociously split for metaphase.

(5) My conclusions in the present paper, made after a study of fertilisation, do not differ in principle from those of Miss Stevens. I state, for my variety of *Culex pipiens*, that parasyn-desis has resulted in actual fusion. Only the assumption that some such fusion has taken place can account for the striking recurrence of three chromosomes in the prophase of somatic tissues in larval, pupal, and imaginal material.

(6) In a preliminary investigation of *Chironomus* sp., an account of which I hope to publish later, I have found a confirmation of the results obtained in *Culex*.

LITERATURE LIST.

1. Dobell and Pringle Jameson (1915).—"The Chromosome Cycle in Coccidia and Gregarines," 'Proc. Roy. Soc.,' vol. lxxxix.
 2. Metz, C. W. (1914).—"Chromosome Studies in the Diptera. I. A preliminary survey of five different types of chromosome groups in the genus *Drosophila*," 'Journ. Exp. Zool.,' xvii.
 3. Stevens, N. M. (1910).—"The Chromosomes in the Germ Cells of *Culex*," 'Journ. Exp. Zool.,' viii.
 4. Taylor, Monica (1914).—"The Chromosome Complex of *Culex pipiens*," 'Quart. Journ. Micr. Sci.' (n.s.), vol. 60.
 5. Woodcock, H. M. (1914).—"On '*Crithidia*' fasciculata in Hibernating Mosquitoes (*Culex pipiens*) and the Question of the Relation of this Parasite with a Trypanosome," 'Zool. Anz.,' Bd. xviii, 8.
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EXPLANATION OF PLATE 20,

Illustrating Miss Monica Taylor's paper on "The Chromosome Complex of *Culex pipiens*.—II: Fertilisation."

All figures were drawn with the Abbe camera to the given scales (Leitz $\frac{1}{12}$ oil imm., Zeiss compensating oculars).

Fig. 1.—Section through egg fixed Gil. Pet. Stained in thionin; differentiated to show "yolk nuclei." "Yolk nuclei" remain deeply stained; chromosomes lose stain when sections are left for some time in absolute alcohol.

Fig. 2.—Section through egg of a raft laid at 4.15 a.m. *ch.* Chorion. *c. m.* Closed micropyle. *f.* Funnel. *s. p. n.* Segmentation resting nucleus surrounded by mass of protoplasm. *v. m.* Vitelline membrane.

Fig. 3.—Resting nuclei; daughter nuclei of fertilisation nucleus; "yolk nuclei" around yolk globules.

Fig. 4.—Prophase of segmentation nucleus; egg one hour old.

Fig. 5.—Prophase.

Fig. 6.—Early prophase.

Fig. 7.—Prophase.

Fig. 8.—Prophase.

Fig. 9.—Early prophase.

Fig. 10.—Metaphase.

Figs. 11 and 12.—Anaphase.

Fig. 13.—Telophase.